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PACIFIC OYSTER EMBRYO RESPONSE TO SEAWATER AND SEDIMENTS FROM THE DUWAMISH RIVER, ELLIOTT BAY, AND CLAM BAY, WASHINGTON

GENERAL APPROACH

The quality of seawater and sediments collected from the upper Duwamish River, Elliott Bay, and Clam Bay, Washington was measured by the Oyster Embryo Bioassay. The development and survival of embryos of the Pacific oyster, Crassostrea gigas, served as the criteria for determining the relative quality of these samples.

METHODS

Sample Collection

Sampling was conducted August 12, 1974. Sediment grab samples were collected with a Van Veen dredge or by hand then placed in 1-gal glass jars having no lids, or 1-gal polyethylene jugs having screw caps. Water samples were collected with a PVC Kemmer water sampler or by hand and held in 1-gal polyethylene jugs having screw caps. All samples were transported in ice chests and iced or refrigerated before being assayed.

Sample Preparation

(Water) Prior to assaying, 1000-ml volumes of sample were poured into each of two, 1-liter polyethylene beakers.

(Sediment Samples) Selected sediment samples were prepared for assaying in the following three ways:

1. Sediment- 10, 1, 0.1, and 0.01 grams of sediment were separately weighed into each of two, 1-liter polyethylene beakers. The contents of the beakers were brought up to 1000 ml with Clam Bay (Control) seawater.

2. Interstitial Water- Sediment was placed in polyethylene containers and centrifuged at 2000 to 8000 RPM for 10 to 50 min at 6 C. The supernate was then filtered through a 0.8 um Millipore HA filter and the filtrate stored in a screw-cap, polyethylene bottle. Prior to assaying,

four decimal dilutions were prepared in duplicate in 1-liter polyethylene beakers by bringing appropriate volumes of interstitial water up to 1000 ml with Clam Bay (Control) seawater.

3. Elutriate Test Filtrate- Sediment was mixed with Elliott Bay surface water in the manner described in the Army Corps of Engineers, Elutriate Test Implementation Guidelines (1974). After settling, the supernate was centrifuged at 8000 RPM for 10 min at 6 C, filtered through a 0.8 um Millipore HA filter, and the filtrate stored in a screw-cap polyethylene bottle. Decimal dilutions were prepared as described above for Interstitial Water.

Bioassay Procedure

Samples were assayed August 13, 1974 by the general oyster embryo technique of Woelke (1972). The adult oysters used in the assay were harvested from Waldrip's June 10, 1974 and conditioned by the Washington State Department of Fisheries Shellfish Laboratory, Brinnon, Washington. Efforts to induce spawning in female oysters were unsuccessful. Eggs were obtained, therefore, by removing the tissue covering the ovary of a shucked oyster, placing the oyster in a beaker of 20 C seawater, and allowing ripe eggs to fall freely into the surrounding seawater. When a sufficient number of eggs had been released, they were fertilized by adding about 5 ml of a stripped sperm suspension. Each 1-liter test and control preparation was inoculated with 2.94×10^4 developing embryos. The cultures were covered with brown paper and air-incubated for 48 hours at 18-22 C.

Sediment assays were terminated by carefully vacuuming the larval culture into a flask without disturbing the settled sediment. Ten milliliters of culture were then transferred to screw-cap glass vials and preserved with 5% formalin. All other assays were terminated by pouring the culture into a 1-liter graduated cylinder, removing 10 ml, and preserving it as described above. Normal and abnormal oyster larvae were enumerated under a microscope at 100X.

Per Cent Abnormal Larvae values were based on the proportion of abnormal larvae to the total number of larvae (normal and abnormal) in each sample. Per Cent Relative Survival values were based on the total number of larvae (normal and abnormal) in each test sample compared with the total number of larvae in the Bioassay Control sample (Clam Bay seawater). Per Cent Net Risk was calculated using the following formula:

$$\text{Per Cent Net Risk} = \frac{\%\ \text{Test Abnormal Larvae} - \%\ \text{Control Abnormal}}{100 - \%\ \text{Control Abnormal}} \times 100$$

Salinity, pH, and dissolved oxygen determinations were performed on selected cultures after termination of the assay.

RESULTS and COMMENTS

The response of Pacific oyster embryos to the seawater and sediments assayed is presented in Table 1.

The 8.02% larval abnormality exhibited by embryos exposed to the Bioassay Control (Clam Bay seawater) exceeded the suggested control abnormality rate of 3%. Consequently, the Per Cent Net Risk statistic was applied to the test results to correct for the "excessive" control abnormality.

It is possible that the termination technique of sampling the entire culture resulted in the inclusion of a number of small abnormal larvae that would have been lost had the recommended practice of sampling just those larvae retained by a 35 um screen been followed. In any event, the assay results should be interpreted with care.

Oyster embryo response was, in most cases, characterized by larval abnormality values that were lower than the control value. Larval abnormalities never exceeded Woelke's (1972) single sample marine water quality criterion of 20% abnormality. In addition, when test results were corrected using the Per Cent Net Risk statistic they also complied with the proposed multiple sample quality criterion of 5% abnormality. These results suggest that all of the samples assayed were of relatively good quality; however, the Per Cent Relative Survival figures seem to present a more meaningful picture. Embryo survival in the test preparations was generally good, but never exceeded embryo survival in the Bioassay Control (Clam Bay seawater). Interestingly, the poorest relative embryo survival (12.8% and 22.8%) was observed in the 10 g/l (wet wt.) preparations of Clam Bay sediment collected at the EPA Manchester Laboratory site. The cause of this poor survival was not determined.

Direct comparison of embryo responses to Duwamish River sediments with those observed by Schink, Westley, and Woelke (1974) was not possible because of the markedly different assay techniques used as well as differences in sampling locations. Embryo response to the Duwamish River (Stations 0-5) Composite sediment sample was, however, quite similar to embryo responses to Duwamish River (Station 2 & Station 4) surface sediments assayed June 6, 1973 (Cummins, 1973).

The assay of interstitial water, directly or via the Elutriate Test, did not offer any toxicological advantage during this series of bioassays. This was particularly significant in the case of interstitial waters obtained directly by centrifugation and filtration. In such cases a sediment wet wt. equivalent of about 200 grams was possible in preparations receiving 100 ml of interstitial water. The apparent "non-toxic" character of the interstitial waters may point to the toxicological importance of the adsorbed, absorbed, and slightly soluable sediment constituents that would have been removed during the rigorous centrifugation and filtration process.

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Table 1. Response of Pacific oyster embryos to water and sediments from the Duwamish River, Elliott Bay, and Clam Bay, Washington.

Number	SAMPLE Location & Type	CONCENTRATION g/l Wet Wt.	CONCENTRATION g/l Dry Wt.[a]	CONCENTRATION ml/l	SALINITY o/oo	pH	OYSTER EMBRYO RESPONSE Mean % Abnormal Larvae	Mean % Net Risk	Mean % Relative Survival
1-10	Clam Bay Seawater (BIOASSAY CONTROL)				28.9	7.9	8.02		100[b]
11-12	Clam Bay Seawater (Carry Along)				28.6	8.0	8.99	1.05	89.4
29-30	Elliott Bay Seawater (0')				27.2	8.0	7.87	0	77.8
31-32	Duwamish River Station 0 Seawater (11')	262+00			26.0	7.9	4.57	0	69.4
33-34	Duwamish River Station 1 Seawater (18')	251+00			27.2	7.8	5.72	0	72.4
35-36	Duwamish River Station 3 Seawater (18')	223+00			27.3	7.9	7.17	0	81.9
37-38	Duwamish River Station 4 Seawater (18')	191+80			27.8	8.0	3.84	0	95.2
39-40	Duwamish River Station 5 Seawater (21')	165+00			27.2	7.9	3.36	0	85.6
41-42	Duwamish River Stations 0-5 Seawater (Composite)				27.5	8.0	4.20	0	82.2

[a] Based on Per Cent Solids determinations performed by EPA Laboratory, Redmond, Washington
[b] Assigned a survival value of 100%

Table 1. Continued

Number	SAMPLE Location & Type	CONCENTRATION g/l Wet Wt.	g/l Dry Wt.[a]	ml/l	SALINITY o/oo	pH	OYSTER EMBRYO RESPONSE Mean % Abnormal Larvae	Mean % Net Risk	Mean % Relative Survival
21-22	Clam Bay Sediment	10			29.3	8.0	0.00	0	12.8
23-24	(Manchester)	1					1.92	0	64.3
25-26		0.1					7.58	0	65.0
27-28		0.01					6.93	0	77.5
13-14	Clam Bay Sediment	10			29.4	7.8	4.95	0	22.8
15-16	(Manchester)	1					4.72	0	78.5
17-18	Carry Along	0.1					8.90	0.96	87.1
19-20		0.01					9.87	2.01	76.5
43-44	Duwamish Sediment	10	7.9		29.4	8.0	4.32	0	78.5
45-46	River Station 0	1	0.79				9.95	2.10	70.9
47-48		0.1	0.079				6.34	0	75.5
49-50		0.01	0.0079				7.38	0	78.0
51-52	Duwamish Sediment	10	5.7		29.5	7.9	4.89	0	65.2
53-54	River Stations 0-5	1	0.57				2.59	0	66.0
55-56	Composite	0.1	0.057				7.34	0	82.7
57-58		0.01	0.0057				6.12	0	84.4
59-60	Duwamish River			10 100%	29.5	8.0	11.9	4.22	93.4
61-62	Station 0			1			7.94	0	89.5
63-64	Interstitial Water			0.1			10.7	2.91	96.9
65-66				0.01			8.35	0.36	88.5
67-68	Duwamish River			100 10%	29.4	8.1	6.04	0	80.5
69-70	Station 1			10			6.55	0	79.2
71-72	Interstitial Water			1			5.42	0	99.6
73-74				0.1			5.04	0	93.5

92.1

88.2

Table 1. Continued

Number	SAMPLE Location & Type	CONCENTRATION g/l Wet Wt.	CONCENTRATION g/l Dry Wt.[a]	CONCENTRATION ml/l	SALINITY o/oo	pH	OYSTER EMBRYO RESPONSE Mean % Abnormal Larvae	Mean % Net Risk	Mean % Relative Survival	
75-76	Duwamish River			100	29.4	8.0	8.24	0.24	82.4	
77-78	Station 3			10			5.95	0	84.4	87.1
79-80	Interstitial Water			1			5.74	0	89.3	
81-82				0.1			7.71	0	92.2	
83-84	Duwamish River			100	29.5	8.0	3.61	0	88.8	
85-86	Station 4			10			5.82	0	79.7	84.9
87-88	Interstitial Water			1			3.62	0	80.7	
89-90				0.1			6.50	0	90.5	
91-92	Duwamish River			100	29.4	8.0	5.53	0	77.5	
93-94	Station 5			10			3.11	0	79.0	2.1
95-96	Interstitial Water			1			2.61	0	83.6	
97-98				0.1			6.20	0	88.1	
99-100	Duwamish River			100	29.4	8.1	3.72	0	73.4	
101-102	Stations 0-5			10			1.03	0	75.1	78.2
103-104	Interstitial Water			1			5.84	0	84.9	
105-106	Composite			0.1			6.18	0	79.2	
107-108	Duwamish River			20 2%	29.5	8.0	4.71	0	83.4	
109-110	Station 0			2			4.21	0	87.3	82.6
111-112	Elutriate Test			0.2			6.83	0	81.0	
113-114	Filtrate			0.02			4.69	0	78.5	
115-116	Duwamish River			200 20%	29.4	8.1	4.76	0	72.8	
117-118	Station 1			20			4.10	0	83.9	81.3
119-120	Elutriate Test			2			3.64	0	92.5	
121-122	Filtrate			0.2			2.32	0	75.8	

Table 1. Continued

Number	SAMPLE Location & Type	CONCENTRATION g/l Wet Wt.	CONCENTRATION g/l Dry Wt.[a]	CONCENTRATION ml/l	SALINITY o/oo	pH	OYSTER EMBRYO RESPONSE Mean % Abnormal Larvae	Mean % Net Risk	Mean % Relative Survival	
123-124	Duwamish River			200	29.3	8.0	6.71	0	86.3	
125-126	Station 3			20			4.64	0	69.2	
127-128	Elutriate Test			2			3.51	0	84.4	79.6
129-130	Filtrate			0.2			4.68	0	78.3	
131-132	Duwamish River			200	29.1	8.0	2.64	0	80.4	
133-134	Station 4			20			2.25	0	73.1	
135-136	Elutriate Test			2			1.57	0	77.8	72.9
137-138	Filtrate			0.2			4.61	0	60.1	
139-140	Duwamish River			200	29.3	8.0	2.28	0	75.5	
141-142	Station 5			20			2.39	0	72.9	
143-144	Elutriate Test			2			1.45	0	84.4	
145-146	Filtrate			0.2			3.09	0	70.4	
147-148	Duwamish River			200	29.4	8.0	5.60	0	78.8	
149-150	Stations 0-5			20			4.70	0	72.8	
151-152	Elutriate Test			2			5.40	0	72.3	73.6
153-154	Filtrate Composite			0.2			3.07	0	70.4	

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